Reticulopodia in testate amoebae (Rhizopodea: Protozoa)

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Summary

The pseudopodial system of *Cryptodifflugia oviformis* and some other testate amoebae consists of numerous filopodia when examined at the resolution of the optical microscope. These filopods are seen to be part of a full reticulum when examined at the ultrastructural level. It is suggested that the general assumption that testate amoebae produce a filopodial system and foraminifera a reticulopodial system may be invalid.

Introduction

The basic difference between lobopod, filopod and reticulopod pseudopodia as found in the class Rhizopodea, Protozoa, is their shape. Lobopodia are blunt extensions of the cytoplasm such as those found in most of naked amoebae and large testate amoebae; filopodia are usually single tapering cytoplasmic extensions as, for example, in *Euglypha* and other small testate amoebae, whilst reticulopodia are normally the granular anastomosing networks of cytoplasm produced by foraminfera. These basic differences are recognized in standard classifications of the rhizopods (e.g. Honigberg *et al.*, 1964; Loeblich & Tappan, 1961, 1964). Most rhizopods produce pseudopodia which are readily assigned to one of these three basic types. Variants of the three types are well known: for example, Page (1975, 1976) illustrates some naked amoebae which produce filose-like pseudopodia and Leidy (1879) in his account of Freshwater Rhizopoda of North America reports a variety of pseudopodial forms in testate amoebae. There is little confusion between the two extreme forms – lobose and reticulose – but in some protozoa either type may merge into the filose type. Greater confusion exists in certain testate amoebae which possess pseudopodia showing a tendency towards both the lobose and reticulose forms.

This account is concerned with observations at the optical and ultrastructural level of the pseudopodial system in *Cryptodifflugia oviformis*, a cosmopolitan soil and moss inhabiting testacean whose general biology, taxonomy and fine structure have been reported previously by Hedley *et al.* (1977).

Materials and methods

A clonal culture of *Cryptodifflugia oviformis* was isolated from the moss, *Eurhynchium praelongum*, by Hedley *et al.* (1977), and formed the working cultures for this study. It is now deposited at the Culture Centre for Algae and Protozoa, The Natural Environment Research Council, Cambridge, England (Reg. No. 1514/2). Live animals from these cultures were examined by both phase-contrast and bright field illumination. For transmission electron microscopy, specimens were fixed for 12 min. in 1% glutaraldehyde in 0·025 M cacodylic acid buffer, followed by 7 min. in 3% glutaraldehyde in the same buffer. After several rinses in the buffer solution, they were post-fixed with 1% osmium tetroxide in distilled water. The material was dehydrated and embedded in Epon 812. Sections cut with a diamond knife on a Porter Blum ultramicrotome were stained with a saturated solution of alcoholic uranyl acetate and Reynold's lead citrate, and examined in an A.E.I. 6B electron microscope operating at 60 kV. The results were recorded on Ilford EM 6 plates.

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Fig. 1 Phase-contrast micrograph of *Cryptodifflugia oviformis* to show the typical arrangement of filopods, ×520.

Results

The pseudopodial system of actively moving specimens of *Cryptodifflugia oviformis* usually consists of several tapering cytoplasmic extensions which may have small branches, and appear to represent a typical filopodial system (Fig. 1). At the ultrastructural level (Fig. 2) the pseudopodial system is seen to consist of the main filopods and many additional cytoplasmic strands, ranging in diameter from 45 nm to 3 μm, which are reminiscent of those seen in foraminifera. If one identifies in such a micrograph as Fig. 2 those pseudopodia which would normally be seen with the optical microscope, that is those whose diameter is greater than 1 μm (Fig. 3), some explanation is needed for the remainder of the cytoplasmic strands in the micrograph. At a higher magnification (Fig. 4) these connecting cytoplasmic strands are similar to those found in foraminifera with a reticulose network (see Figs. 8 and 9).

A model of the type of pseudopodial arrangement which would be consistent with the micrograph (Figs. 2 and 3) is presented in Figs 5 and 6. This represents only one of many possible arrangements of the pseudopodia which would correspond to such a micrograph. The contraction of the filopods as depicted in Fig. 5 is what one would expect, and does observe when an animal with an extruded filopodial system is immersed and subsequently fixed in glutaraldehyde.

Discussion

In describing the biology and ultrastructure of *Cryptodifflugia oviformis*, Hedley *et al.* (1977) stated that previous studies, among which de Saedeleer (1932) may be mentioned, had drawn attention to the fact that the pseudopodia appeared to be intermediate in form between lobose

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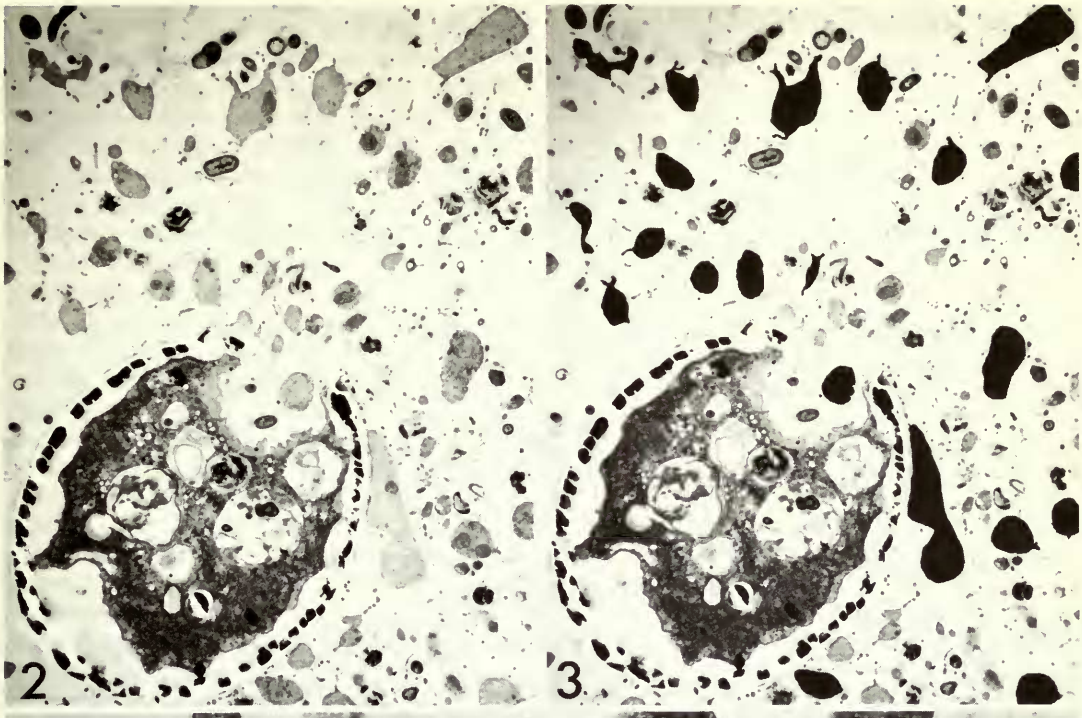
Fig. 2 Section of a specimen of *C. oviformis* to illustrate the range of pseudopodial structures visible at the ultrastructural level, ×4300.

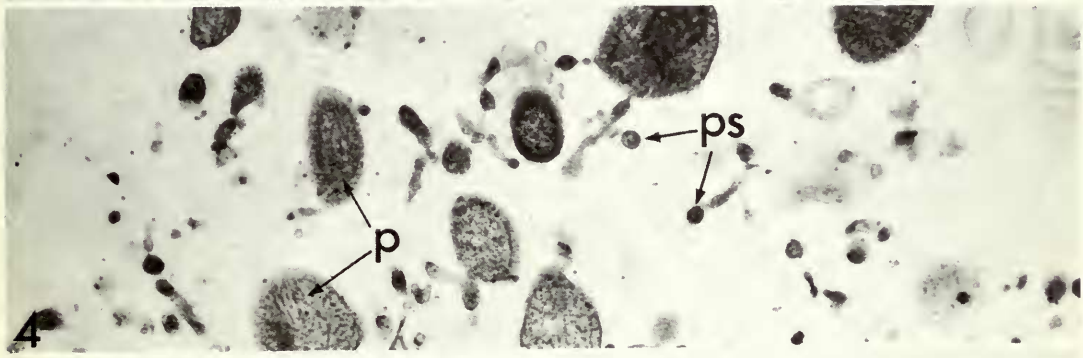
Fig. 3 Section as in Fig. 2 showing those pseudopodia with a diameter greater than 1 μm masked in black, ×4300.

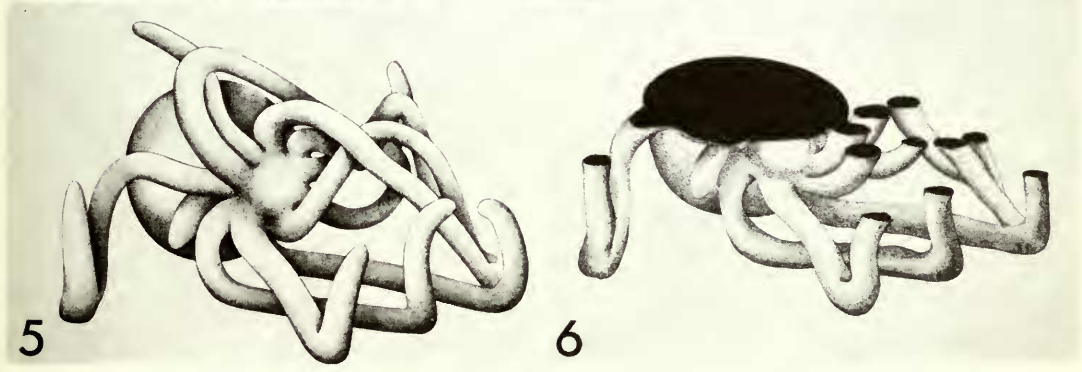
Fig. 4 Section of pseudopodia (p) and pseudopodial strands (ps) near the aperture of *C. oviformis*, ×21 000.

Fig. 5 A model of a specimen of *C. oviformis* to correspond with the micrographs shown in Figs 2 and 3.

Fig. 6 Diagram to show model cut in the same plane as the sections in Figs 2 and 3.


2
3


ps
p
4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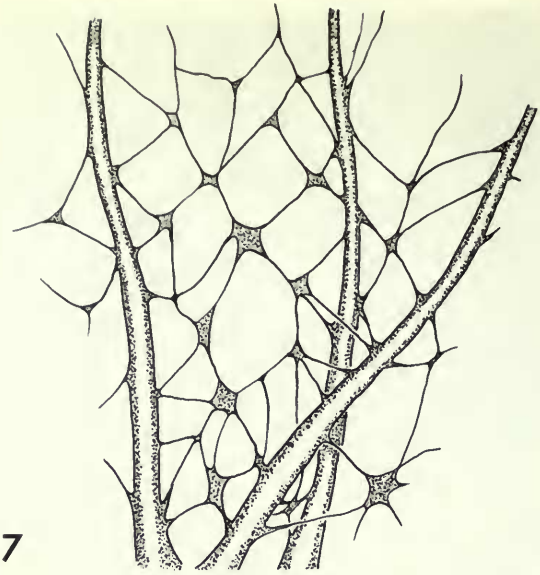
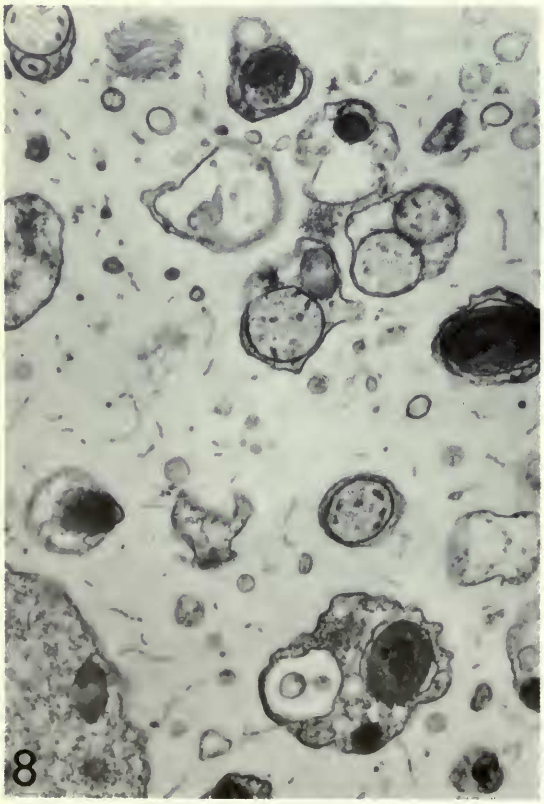


Fig. 7 Diagram showing a proposed pseudopodial network for *C. oviformis*, based on the examination of several micrographs.



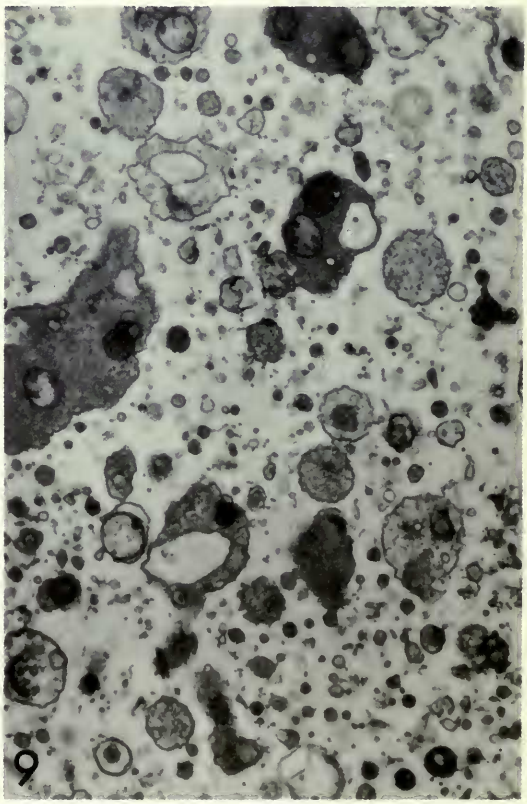


Fig. 8 Section of the pseudopodial network of *Allogromia laticollaris* (Foraminifera), ×30 000.
Fig. 9 Section showing the size range of pseudopodial structures in *Shepheardella taeniformis* (Foraminifera), ×15 000.

and filose structures. We did not comment on them at the time, as there was insufficient information on the ultrastructural organization in other testate amoebae. It is now suggested that in *C. oviformis* the pseudopodial system is an anastomosing network. This appears to be so in other forms possessing filose pseudopodia, for example, *Euglypha* and *Trinema*, and also for some with lobose pseudopodia, for example, *Arcella*, *Centropyxis* and *Nebela* (unpublished observations). If this proves to be generally true of testate amoebae then the distinction between a filopodial system in such rhizopods and a reticulopodial system in foraminifera ceases to be valid. The difference remaining between the two groups would then be whether or not the network in testate amoebae is granular, as it clearly is in the foraminifera when viewed with the optical microscope (Hedley, 1964). At present the only report of granular filose pseudopodia in testate amoebae is that of Berrend (1966) who described the bi-directional flow of granules in *Cyphoderia ampulla*.

The ultrastructure of the pseudopodia of *C. oviformis* (Fig. 4) compares well with our previous studies of the fine structure of foraminifera, such as *Shepheardella taeniformis* (Fig. 9), *Allogromia laticollaris* (Fig. 8), *Iridia diaphana* and *Boderia turneri*, and with descriptions of foraminiferal pseudopodia by other workers (Wohlfarth-Botterman, 1961; Lengsfeld, 1969; Marsalek, 1969; Schwab, 1969; Febvre-Chevalier, 1971; Anderson & Bé, 1976, 1978).

The range in diameters of the pseudopodia in *C. oviformis* is 45 nm–3 μm and this compares favourably with those we have observed in foraminifera – *Allogromia laticollaris* 30 nm–2 μm, *Boderia turneri* 40 nm–1·5 μm, and *Iridia diaphana* 40 nm–2 μm – and those reported previously for the benthic foraminifer *Shepheardella taeniformis* 30 nm–1 μm (Hedley *et al.*, 1967) and the planktonic foraminifer *Globigerinoides sacculifera* 80 nm–2 μm (Anderson & Bé, 1978).

In conclusion it is suggested that the basic architecture of pseudopodial fine structure in testate amoebae supports the view that these forms produce a reticulopodium. In many characteristics they are similar to the reticulopodia found in foraminifera except that in the foraminifera the pseudopodia are invariably granular and exhibit bi-directional streaming when observed with the optical microscope.

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